

**PCT**WORLD INTELLECTUAL PROPERTY ORGANIZATION  
International Bureau

## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<b>(51) International Patent Classification <sup>6</sup>:</b> <b>C12Q 1/68, C12N 15/31, C07K 14/21, 16/12</b>	<b>A3</b>	<b>(11) International Publication Number:</b> <b>WO 97/01647</b> <b>(43) International Publication Date:</b> 16 January 1997 (16.01.97)
<b>(21) International Application Number:</b> PCT/US96/11132 <b>(22) International Filing Date:</b> 28 June 1996 (28.06.96) <b>(30) Priority Data:</b> 60/000,828 28 June 1995 (28.06.95) US <b>(71) Applicant:</b> TRUSTEES OF HEALTH & HOSPITALS OF THE CITY OF BOSTON, INC. [US/US]; 6th floor, 1010 Massachusetts Avenue, Boston, MA 02119 (US). <b>(72) Inventor:</b> GOLDSTEIN, Richard; 12 Howland Street, Cambridge, MA 02138 (US). <b>(74) Agents:</b> PIERRI, Margaret, A. et al.; Fish & Neave, 1251 Avenue of the Americas, New York, NY 10020 (US).	<b>(81) Designated States:</b> AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).  <b>Published</b> <i>With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>  <b>(88) Date of publication of the international search report:</b> 01 May 1997 (01.05.97)	
<b>(54) Title:</b> DNA SEQUENCES FOR IDENTIFYING HIGHLY TRANSMISSIBLE LINEAGES OF PSEUDOMONAS (BURKHOLDERIA) CEPACIA  <b>(57) Abstract</b> <p>The present invention provides DNA based fingerprints and DNA sequences for indentifying highly transmissible lineages of <i>Pseudomonas cepacia</i>. More specifically, the present invention provides genetic band patterns or DNA based fingerprints for identifying highly transmissible lineages of <i>Pseudomonas cepacia</i> produced by: (a) ribotyping analyses, i.e., the determination of restriction fragment length polymorphisms (RFLPs) associated with the multicopy RNA operon (rm); and (b) pulsed field gel electrophoresis (PFGE)-based resolution of chromosomal macro-RFLP patterns. Also provided are unique primer oligonucleotide sequences and DNA probes derived from variants of a gene, encoding a 17-KDa major subunit pilin protein (cbIA) of a cystic fibrosis-associated <i>Pseudomonas cepacia</i>. The invention also discloses methods and diagnostic kits for identifying highly transmissible lineages of <i>Pseudomonas cepacia</i> that utilize the above DNA based fingerprints, oligonucleotide primers and DNA probes. Another object of this invention is to provide isolated DNA molecules encoding a 17-KDa major subunit pilin protein of certain cystic fibrosis-associated <i>Pseudomonas cepacia</i> strains, as well as recombinant DNA molecules, transformed hosts and methods for the production of that protein. Also contemplated are antibodies to the 17-KDa major subunit pilin protein.</p>		

**FOR THE PURPOSES OF INFORMATION ONLY**

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AM	Armenia	GB	United Kingdom	MW	Malawi
AT	Austria	GE	Georgia	MX	Mexico
AU	Australia	GN	Guinea	NE	Niger
BB	Barbados	GR	Greece	NL	Netherlands
BE	Belgium	HU	Hungary	NO	Norway
BF	Burkina Faso	IE	Ireland	NZ	New Zealand
BG	Bulgaria	IT	Italy	PL	Poland
BJ	Benin	JP	Japan	PT	Portugal
BR	Brazil	KE	Kenya	RO	Romania
BY	Belarus	KG	Kyrgyzstan	RU	Russian Federation
CA	Canada	KP	Democratic People's Republic of Korea	SD	Sudan
CF	Central African Republic			SE	Sweden
CG	Congo	KR	Republic of Korea	SG	Singapore
CH	Switzerland	KZ	Kazakhstan	SI	Slovenia
CI	Côte d'Ivoire	LI	Liechtenstein	SK	Slovakia
CM	Cameroon	LK	Sri Lanka	SN	Senegal
CN	China	LR	Liberia	SZ	Swaziland
CS	Czechoslovakia	LT	Lithuania	TD	Chad
CZ	Czech Republic	LU	Luxembourg	TG	Togo
DE	Germany	LV	Latvia	TJ	Tajikistan
DK	Denmark	MC	Monaco	TT	Trinidad and Tobago
EE	Estonia	MD	Republic of Moldova	UA	Ukraine
ES	Spain	MG	Madagascar	UG	Uganda
FI	Finland	ML	Mali	US	United States of America
FR	France	MN	Mongolia	UZ	Uzbekistan
GA	Gabon	MR	Mauritania	VN	Viet Nam

DNA SEQUENCES FOR IDENTIFYING HIGHLY TRANSMISSIBLE LINEAGES OF  
PSEUDOMONAS (BURKHOLDERIA) CEPACIA

FIELD OF THE INVENTION

5           This invention relates generally to the detection of highly transmissible strains of *Pseudomonas (Burkholderia) cepacia*,\* and particularly to the use of DNA based fingerprints and sequences for identifying such epidemic lineages.

10           BACKGROUND OF THE INVENTION

*Pseudomonas cepacia* is an aerobic, gram-negative bacillus with an ubiquitous distribution in soil and water. In recent years this organism has emerged as an important pathogen among cystic fibrosis  
15 (CF) patients. CF patients with respiratory colonization or infection with *P. cepacia* have higher morbidity and mortality than those CF patients not infected by this organism.<sup>1-6</sup> While the significant increase in *P. cepacia* infection suggests epidemic  
20 spread<sup>3, 6-8</sup>, the source and transmissibility of

---

\* In view of recent molecular phylogenetic analyses, the bacterium *Pseudomonas cepacia* has been renamed *Burkholderia cepacia*. However, in order to be consistent with the terminology of the literature cited in the specification, the original nomenclature is maintained herein.

- 2 -

*P. cepacia* remains controversial<sup>9</sup>. Nonetheless, given the potentially grave consequence of *P. cepacia* infection, stringent infection control policies have been adopted, many CF camps in North America have been  
5 closed and all but one lung transplant center have ceased to accept *P. cepacia*-infected CF patients as transplant candidates.

The epidemiology of *P. cepacia* infection has been previously examined by both ribotyping<sup>1, 9</sup> and  
10 pulsed-field gel electrophoresis (PFGE)-based resolution of chromosomal macro-restriction fragment profiles<sup>3, 9, 10</sup>. Comprehensive studies applying both methods generated two very different conclusions regarding clonality, persistence, and transmissibility.  
15 One study in the UK (Western General Hospital, Edinburgh), found a clonal relationship among isolates from 13 patients over six years<sup>3</sup>. In contrast, during an eight year period at a US CF center (UNC Hospitals, Chapel Hill), not a single identical or closely-related  
20 strain was found among 23 infected clinic and lung transplant patients<sup>9</sup>. Serial isolate analysis further confirmed this picture, typically demonstrating persistent infection by a single strain.

There also existed an isolate collection from  
25 another CF center (Hospital for Sick Children, Toronto) where there was anecdotal evidence for an epidemic of *P. cepacia*. Although the isolates were not characterized for genetic-relatedness, they had been uniformly resolved to express peritrichous, giant cable  
30 (Cbl)-like pili that specifically bound to CF mucin and airway epithelial cells<sup>4, 11</sup>.

As the *cbIA* pilin subunit gene encoded by all 15 of the Toronto isolates was the first adhesin pilus

SUBSTITUTE SHEET (RULE 26)

- 3 -

subunit gene characterized for *P. cepacia*, a subsequent hybridization-based survey for the presence of *cblA* was carried out on multiple isolates from seven other CF centers in the US (Chapel Hill NC, Jackson MS, Norfolk VA, Cleveland OH, Phila. PA, New York NY) and Europe (Edinburgh) as well as clinical and environmental strains. All of these isolates were *cblA*<sup>-</sup> except for one isolate from a CF center in Jackson Mississippi.

By examining the genetic-relatedness of *cblA*<sup>+</sup> and *cblA*<sup>-</sup> strains, we have discovered the emergence of a highly transmissible lineage, seemingly adapted for efficient transmission in the CF population. Resolved genetic markers uniquely associated with this lineage, which may be used to rapidly identify its presence, are of immediate practical importance in CF centers in both Europe and North America.

#### SUMMARY OF THE INVENTION

It is an object of this invention to provide chromosomal restriction fragment length polymorphism (RFLP) patterns (generically referred to as DNA based fingerprints) and DNA sequences for identifying highly transmissible lineages of *Pseudomonas cepacia*.

More specifically, the present invention provides DNA based fingerprints for identifying highly transmissible lineages of *Pseudomonas cepacia* produced by: (a) ribotyping analyses, i.e., the determination of RFLPs associated with the multicopy RNA operon (*rrn*); and (b) pulsed field gel electrophoresis (PFGE)-based resolution of chromosomal macro-RFLP patterns.

Another object of this invention is to provide isolated DNA molecules encoding a 17-KDa major

- 4 -

subunit pilin protein (cblA) of cystic fibrosis-associated, epidemic and non-epidemic *Pseudomonas cepacia* strains. Also contemplated is the recombinant production of polypeptides using the isolated DNA molecules. The recombinant polypeptides are then used to produce antibodies for use in methods for identifying epidemic strains of *Pseudomonas cepacia*.

It is also an object of this invention to provide unique primer oligonucleotide sequences derived from cblA gene variants for use in polymerase chain reaction (PCR)-based methods for detection of highly transmissible strains of *Pseudomonas cepacia*.

It is also an object of this invention to provide DNA probes derived from unique regions of variant cblA gene sequences for detection of highly transmissible strains of *Pseudomonas cepacia*.

It is also an object of this invention to provide methods for identifying highly transmissible lineages of *Pseudomonas cepacia* that utilize the above DNA based fingerprints, oligonucleotide primers and DNA probes.

It is a further object of this invention to provide diagnostic kits for identifying highly transmissible strains of *Pseudomonas cepacia*.

- 5 -

Brief Description of the Drawings

Figs 1 A-C together depict RFLPs of 17 *P. cepacia* isolates cited in the specification. Lane order for the 17 isolates is maintained in all three figures. Isolate numbers of examined strains appear at the top of the figure immediately above each lane. Subscript letters preceding isolate number indicate CF center from which *P. cepacia* (PC) strain was isolated: PC<sub>E</sub>, Edinburgh Scotland; PC<sub>MS</sub>, Jackson MS; PC<sub>NC</sub>, Chapel Hill NC; PC<sub>NY</sub>, New York NY; PC<sub>OH</sub>, Cleveland OH; PC<sub>T</sub>, Toronto Canada, and PC<sub>VA</sub>, Norfolk VA.

Fig. 1A.: PFGE-resolved *SpeI* RFLPs. As described previously<sup>9</sup>, samples were prepared and restriction fragments separated by pulsed field gel electrophoresis with a CHEF Mapper system (BIO-RAD) through 1% agarose using a field strength of 6 V/cm and an initial and final pulse time of 1.2 sec and 54 sec, respectively. Fragment sizes were determined using a  $\lambda$  concatenate ladder. Bar-code format translation of chromosomal fingerprint profiles was made using a Macintosh Quadra 950 running Gene Construction Kit (Texto). Fragments below 100 kbp are not shown. In the latter range, Toronto and Edinburgh isolates displayed in lanes 1-8 had two identical fragments (60 kbp and 48 kbp). Other isolates (lanes 9-17) had polymorphic sets of three to six fragments in this lower range.

Fig. 1B: *rrn* *EcoRI* RFLPs. Southern blot hybridization methods were as we described previously<sup>9, 26</sup> using a <sup>32</sup>P-labeled *rrnB* probe spanning the entire *rrnB* operon of *E. coli* K12.

- 6 -

Fig. 1C: *cblA* hybridization analysis of *EcoRI* generated RFLPs. This was accomplished by stripping bound *rrn* probe from the membrane used in Fig. 1B, followed by hybridization with a previously described *cblA* gene probe<sup>4</sup> using standard methods<sup>26, 27</sup>.

Fig. 2: *rrn*-RFLP based phylogenetic tree of representative isolates from patients at seven CF centers in North America (Chapel Hill NC, Jackson MS, Norfolk VA, Cleveland OH, Philadelphia PA, New York NY, Toronto Ontario) and Europe (Edinburgh) plus environmental and clinical (non-CF) sources. All cited isolates are described in the text and in the Methods section. Indicated isolate number is followed by source (CF, environmental or clinical). *cblA*<sub>1</sub><sup>+</sup>, *cblA*<sub>2</sub><sup>+</sup>, isolate(s) that encode the *cblA* gene (Fig. 1C) and express adhesin Cbl pili (Fig. 3). *cblA*<sub>1</sub><sup>+</sup>, identical 501 bp sequence carried by Toronto and Edinburgh CF center isolates (Fig. 4); *cblA*<sub>2</sub><sup>+</sup>, polymorphic 501 bp sequence carried by Jackson Mississippi CF center isolate PC<sub>MS</sub>-2323 (Fig. 4). Number above each branch indicates the percentage of time each was joined together under bootstrap analysis<sup>14</sup> (confidence intervals less than 10 have been omitted for clarity). The lineages included in this tree are representative of the larger sample of isolates collected. Multiple CF patient serial isolates of an identical *rrn* RFLP profile have not been included as they do not affect the tree topology. However, multiple isolates from Toronto (PC<sub>T</sub>-5, PC<sub>T</sub>-7) and Edinburgh (PC<sub>E</sub>-1359, PC<sub>E</sub>-2315) CF centers are noted because further analysis by DNA sequence revealed that

**SUBSTITUTE SHEET (RULE 26)**

- 7 -

the *cblA* genes encoded by these four isolates are identical (Fig. 4). The remaining 13 and 11 isolates, respectively, from each of these two CF centers are members of the indicated epidemic lineage based on 100% correlation of their *rrn* RFLP profiles with those of the prototypic patterns of the Toronto/Edinburgh isolates shown in Fig. 1B.

Fig. 3: Transmission electron micrograph of Toronto epidemic strain PC<sub>T</sub>-7 expressing Cbl adhesin pili. High resolution was achieved with a JOEL 100 CX electron microscope as previously described<sup>11</sup>. Bar in lower right corner, 0.1  $\mu$ m.

Fig. 4: Identical 501 bp sequence (top-most line) of the *cblA* structural gene encoded by 2 prototypic Toronto epidemic isolates (PC<sub>T</sub>-7 and PC<sub>T</sub>-5) and 2 prototypic Edinburgh isolates (PC<sub>E</sub>-2315 and PC<sub>E</sub>-1359) compared to the variant *cblA* sequence carried by the single Jackson Mississippi CF center isolate PC<sub>MS</sub>-2323 (lower line).

## 20 DETAILED DESCRIPTION OF THE INVENTION

One aspect of the present invention is directed to the identification of highly transmissible strains of *Pseudomonas cepacia* using DNA based fingerprints. More specifically, such identification

- 8 -

- is accomplished by the use of fingerprints produced by:
- (a) ribotyping analyses, i.e., the determination of restriction fragment length polymorphisms (RFLPs) associated with the multicopy RNA operon (*rrn*); and
  - 5 (b) pulsed field gel electrophoresis (PFGE)-based resolution of chromosomal macro-RFLP patterns.

- The techniques for ribotyping are known to those of skill in the art. Generally, DNA samples from bacterial strains are isolated, digested with a
- 10 restriction endonuclease such as *EcoRI* and separated by agarose gel electrophoresis. The DNA fragments from the agarose gel are then transferred to nitrocellulose membranes and probed with either radiolabeled or chemiluminescent *E. coli* ribosomal RNA (*rRNA*) probes.
- 15 The RFLPs detected by hybridization with the *E. coli* *rRNA* probes are then analysed to categorize the bacterial strains according to their distinctive bands of *rRNA* encoding DNA (i.e., "DNA fingerprints").

- Similarly, the skilled artisan would also be
- 20 familiar with the published methods for PFGE-based resolution of chromosomal macro-RFLP patterns. Such methods involve immobilization of bacteria in agarose plugs after which the bacteria are lysed and the DNA is digested with an infrequently (rare) cutting
- 25 restriction endonuclease such as *SpeI*. The agarose plugs with the digested DNA are then subjected to transverse alternating field electrophoresis in agarose gels using commercially available equipment. The gels are generally stained with ethidium bromide or cyber-
- 30 green and the resulting chromosomal RFLPs are analysed preferably after computer-generated translation of the RFLP profiles into bar code format using commercially available equipment and software.

**SUBSTITUTE SHEET (RULE 26)**

- 9 -

Also well known to those skilled in the art are the standard criteria for the statistical analysis of the ribotypes and the chromosomal RFLP profiles obtained using the above methods. Using these  
5 statistical criteria, bacterial strains may be compared and their genetic relationships characterized. In the present invention, the determination of whether a given strain of *Pseudomonas cepacia* is highly transmissible is made by comparing its ribotypic and chromosomal RFLP  
10 profiles to those of a known strain of highly transmissible lineage.

In another aspect of the present invention, isolated DNA molecules encoding a 17-kDa major subunit pilin protein (cblA) of cystic fibrosis-associated,  
15 epidemic and non-epidemic *Pseudomonas cepacia* strains are provided. The genes encoding the cblA protein are isolated and sequenced by standard techniques. The cblA genes isolated from epidemic and non-epidemic strains of *Pseudomonas cepacia* enabled for the first  
20 time the comparison of those variant genes and the identification of the differences in their nucleotide sequences.

The isolated DNA molecules are then used for the recombinant production of the 17-kDa major subunit pilin protein (cblA) of cystic fibrosis-associated,  
25 epidemic and non-epidemic *Pseudomonas cepacia* strains. As such, the present invention also contemplates recombinant DNA molecules containing the above DNA molecules and unicellular hosts transformed with those  
30 recombinant DNA molecules. The recombinant polypeptides and their fragments are then used for the production of antibodies that can distinguish epidemic and non-epidemic strains of *Pseudomonas cepacia* in

- 10 -

standard immunologic assays such as ELISA,  
radioimmunoassay and western blots. The methods for  
recombinant protein production, protein purification  
and generation of antibodies are well within the  
5 purview of the ordinary skilled artisan.

This invention also provides unique primer  
oligonucleotide sequences derived from *cblA* gene  
variants for use in polymerase chain reaction (PCR)-  
based methods for detection of highly transmissible  
10 strains of *Pseudomonas cepacia*. These unique primers  
are usually synthesized using standard procedures  
following identification of desirable nucleotide  
sequences based on the comparison of the *cblA* gene  
sequences of epidemic and non-epidemic strains of  
15 *Pseudomonas cepacia*. The PCR techniques contemplated  
by and used in the present invention are well known.  
Essentially, the primers, DNA from the bacterial strain  
to be tested and a thermostable PCR enzyme are mixed  
and the reaction carried out according to established  
20 procedure in a thermocycler block. The PCR products  
are then analyzed generally by electrophoretic  
separation.

The invention also provides DNA probes  
derived from unique regions of variant *cblA* gene  
25 sequences that may be used in standard hybridization  
based assays such as colony hybridization or Southern  
blot transfers to detect highly transmissible strains  
of *Pseudomonas cepacia*.

The present invention also contemplates the  
30 use of the above DNA based fingerprints,  
oligonucleotide primers, DNA hybridization probes,  
polypeptides and antibodies in diagnostic kits for the

**SUBSTITUTE SHEET (RULE 26)**

- 11 -

detection of highly transmissible lineages of *Pseudomonas cepacia*.

In order that this invention may be better understood, the following examples are set forth.

- 5 These examples are for purposes of illustration only, and are not to be construed as limiting the scope of the invention in any manner.

### EXAMPLES

#### Materials and Methods

10 **Bacterial isolates:**

- 133 *Pseudomonas cepacia* isolates were obtained from the following sources: (i) sixty-five isolates from patients at the University of North Carolina Cystic Fibrosis Center (1985 through 1993) including 17 clinic and 5 transplant patients, 4 of whom were infected transfers from other distant locations as described previously<sup>9, 11</sup>. Those cited in the Figures include isolates PC<sub>NC</sub>-566, PC<sub>NC</sub>-1711, PC<sub>NC</sub>-1845, PC<sub>NC</sub>-1910, PC<sub>NC</sub>-1948, PC<sub>NC</sub>-2008, PC<sub>NC</sub>-2028, PC<sub>NC</sub>-2211, PC<sub>NC</sub>-2225 and PC<sub>NC</sub>-2747, each from different local clinic patients, and four isolates from infected transfer patients (PC<sub>NY</sub>-792 from New York, New York, PC<sub>VA</sub>-1963 from Norfolk Virginia, PC<sub>OH</sub>-2034 from Cleveland Ohio and the *cbIA*<sup>+</sup> isolate PC<sub>MS</sub>-2323 from Jackson Mississippi); (ii) 8 ATCC clinical and environmental isolates: human endocarditis PC<sub>ATCC</sub>-13945, human urinary tract PC<sub>ATCC</sub>-17765, human bronchial PC<sub>ATCC</sub>-25609, human tibia fracture PC<sub>ATCC</sub>-27515, forest soil (Trinidad) PC<sub>ATCC</sub>-17759, onion PC<sub>ATCC</sub>-25416 (the ATCC *P. cepacia* type strain), soil (California) PC<sub>ATCC</sub>-29352, cornfield soil (New Jersey)

- 12 -

- PC<sub>ATCC</sub>-39277, all as described previously<sup>9, 11</sup>; (iii) 15 *cblA*<sup>+</sup> isolates from 15 CF center clinic patients at the Hospital for Sick Children (1987 through 1988), Toronto. Those cited in the Figures include isolates
- 5 PC<sub>T</sub>-5, PC<sub>T</sub>-7, PC<sub>T</sub>-19, PC<sub>T</sub>-25<sup>4, 11</sup>; (iv) two isolates, PC<sub>E</sub>-SBC27 and PC<sub>E</sub>-SBC29, from two CF patients at Western General Hospital (Edinburgh Scotland) that were not associated with epidemic transmission within this CF center<sup>3, 4, 11</sup>; (v) four isolates from clinic
- 10 patients at Western General Hospital (1989 through 1990), Edinburgh, all which had been associated with epidemic transmission within this CF center<sup>3</sup>. Those cited in the Figures include isolates PC<sub>E</sub>-509, PC<sub>E</sub>-1359, PC<sub>E</sub>-1392 and PC<sub>E</sub>-2315; (vi) ten isolates from 10
- 15 patients at a Philadelphia Pennsylvania CF center<sup>2</sup>. Those cited in the Figures include isolates PC<sub>PA</sub>-535, PC<sub>PA</sub>-542 and PC<sub>PA</sub>-544; (vii) twenty-four isolates from 24 patients at the Rainbow Babies and Children's Hospital, Cleveland Ohio<sup>17</sup> including cited isolate
- 20 PC<sub>OH</sub>-524, PC<sub>OH</sub>-525 and PC<sub>OH</sub>-2034; and (viii) 5 bronchial isolates from 5 ventilator patients at Boston City Hospital (Massachusetts) including cited isolate PC<sub>MA</sub>-3137.

#### Restriction Fragment Length Polymorphism Analyses

##### 25 Ribotyping:

- Chromosomal DNA was prepared using the following procedure. Overnight cultures were diluted 10-fold in 10 ml of LB and incubated at 37°C until they reached mid-log phase. Cells were pelleted, washed
- 30 twice with 0.9% NaCl, and then resuspended in cold TE (10 mM Tris-HCl, 10 mM EDTA [pH 8.0]). Lysozyme was added to a final concentrations of 20 mg/ml, and the

**SUBSTITUTE SHEET (RULE 26)**

- 13 -

solution was incubated at 37°C for 30 min. Proteinase K (20 mg/ml in TE; Sigma) and sodium dodecyl sulfate were added to final concentration of 20 µg/ml and 0.1%, respectively. The lysates were incubated overnight at 50°C. Sarcosine-free acid (Sigma) was added to a final concentration of 2%, and the solution was mixed gently. The DNA was then purified by cesium chloride-ethidium bromide equilibrium density gradient centrifugation.<sup>20</sup>

*EcoRI* *rRNA* (*rrn*) restriction fragment length polymorphisms (RFLPs) for phylogenetic analyses were analyzed by using an *rrnB* probe spanning the entire *rrnB* operon of *Escherichia coli* K-12.<sup>28</sup> Plasmid DNA was purified by the alkaline lysis method<sup>29</sup>, followed by cesium chloride-ethidium bromide gradient centrifugation.<sup>20</sup> The DNA restriction fragment used to generate the probe was separated by horizontal slab gel electrophoresis in 0.8% low-melting point agarose. The relevant restriction fragment from a slice of the gel was radiolabeled in the agarose by random oligonucleotide priming with [ $\alpha$ -<sup>32</sup>P]dCTP (Dupont, NEN Research Products).<sup>30</sup>

The chromosomal DNA prepared according to the above-described method was restricted with *EcoRI* and the fragments were separated by agarose gel electrophoresis. Following electrophoresis, restriction fragments were transferred to nitrocellulose membranes and hybridized with the *rrnB* probe described above. Hybridized bands were visualized by autoradiography and the banding patterns were analysed using standard methods.<sup>9,26</sup>

Pulsed-field gel electrophoresis (PFGE):

- 14 -

Cells were grown to early log phase in LB, harvested by centrifugation, washed with 1:1 TE buffer, resuspended in 1 ml of 10:1 TE buffer, and mixed with 1.2 volumes of melted 1% InCert agarose (Bio-Rad) in TE buffer. The mixture was dispensed into 120- $\mu$ l insert molds (Pharmacia) and allowed to solidify on ice. Plugs were sliced and incubated in 20  $\mu$ g of lysozyme per ml at 37°C for 12 h. The lysozyme buffer was replaced with ESP buffer (0.5 MEDTA [pH9], 1% sarcosyl, 200  $\mu$ g of proteinase K [Sigma] per ml), and the plugs were incubated at 37°C for 5 h and then washed with TE buffer for 4 h at 37°C. Single plug slices were incubated with *SpeI* (Boehringer Mannheim) in restriction enzyme buffer for 2 h. Restriction fragments were separated by PFGE using a CHEF Mapper system (Bio-Rad) through 1% agarose (Bio-Rad; Molecular Biology Certified grade) in 0.5 X TBE (1 X TBE is 0.9 M Tris-HCl, 0.9 M boric acid, and 1.0 mM EDTA). Electrophoresis was performed for 24h in 0.5 X TBE buffer at 14°C, with a field strength of 200 V (6V/cm) and initial and final pulse times of 1.2 and 54 s, respectively. Lambda concatemers were used as DNA size standards. The gels were stained with ethidium bromide or cyber-green and photographed under UV transillumination with a Polaroid camera. Fragment sizes were determined, and the computer-generated translation of chromosomal fingerprint profiles was made into bar code format by using a Macintosh Quadra 950 and Gene Construction Kit (Texto) software.

30 Statistical Analysis of Ribotypes and PFGE Patterns:  
According to established criteria for *P. cepacia*<sup>24</sup>, strains were assigned to the same ribotype

**SUBSTITUTE SHEET (RULE 26)**

- 15 -

when comparison of sizes of hybridizing fragments revealed 3 or fewer bands differing between the two patterns under comparison.

Standard criteria were used for comparing PFGE chromosomal fingerprints.<sup>23</sup> Briefly, PFGE patterns were considered different (i) when they had the same number of DNA fragments but when the size of at least one band varied by more than 1 standard deviation (5%), (ii) when they exhibited a different number of DNA fragments, or (iii) when the sum of the sizes of the differing bands in the first PFGE pattern did not correspond to that of the differing DNA fragments in the second PFGE pattern.

Quantitative pairwise comparison of both types of RFLP patterns was accomplished using the Dice coefficient of similarity calculated as  $D = 2n_{xy}/(n_1 + n_2)$ , where  $n_1$  is the total number of DNA fragments from strain X,  $n_2$  is the total number from strain Y, and  $n_{xy}$  the number of fragments identical in the two strains<sup>12, 25</sup>. The coefficient of similarity for two PFGE RFLPs  $D \geq 0.90$  represents closely related strains, while unrelated strains have  $D \leq 0.60$ . Intervening values, remarkably, were not observed, and values between 0.5 and 0.6 are rare<sup>9</sup>. For *rrn* RFLPs, given that *P. cepacia* strains typically display 7-10 distinct hybridizing bands, the shared ribotype (above) would correspond to  $D=0.79$  to  $0.85$ . Comparisons between mean values were performed by Student's *t* test using a Systat program (Systat Inc.).

#### 30 Amplification and Sequencing of the *cblA* Gene:

Using previously described methods<sup>9</sup>, CsCl equilibrium density gradient purified chromosomal DNA<sup>20</sup> was isolated from the two prototypic Toronto epidemic

- 16 -

isolates (PC<sub>T</sub>-7 and PC<sub>T</sub>-5), the two prototypic Edinburgh epidemic isolates (PC<sub>E</sub>-2315 and PC<sub>E</sub>-1359) and the single Jackson Mississippi CF center isolate PC<sub>MS</sub>-2323. From each of these *cblA* probe-positive chromosomes, the *cblA* gene was PCR amplified<sup>21</sup> using a DNA thermocycler (Perkin-Elmer) with a GeneAmp PCR Core Reagents Kit (*ibid.*). Based on the previously determined sequence of the *cblA* gene encoded by isolate PC<sub>T</sub>-74, sense and antisense primers used for these reactions were 5'-  
10 CCAAAGGACTAACCCA-3' and 5'ACGCGATGTCCATCACA-3', respectively. PCR reactions were: cycle-1, 2 min at 94°C, 2 min at 37°C, 1 min at 72°C. The remaining 29 cycles were: 1 min at 94°C, 1 min at 45°C, 1 min at 72°C, followed by 7 min extension at 72°C. PCR products  
15 were electrophoresed through 0.8% agarose and for each a single band was observed with ethidium bromide staining. Bands were electroeluted into DEAE membrane (Schleicher & Schuell) and cloned with a TA Cloning Kit (Invitrogen). DNA sequences were determined by the  
20 Sanger dideoxy method<sup>22</sup> with the same primers used for PCR amplification (above). Five PCR-amplified *cblA* gene clones of PC<sub>MS</sub>-2323 were generated, three of which were sequenced for confirmatory purpose, with no variation resolved.

**SUBSTITUTE SHEET (RULE 26)**

PCR Amplification Detection, Using Unique Primer Oligonucleotide Sequences Derived From *cbLA* Gene Variants:

5 This method was based upon three 'sets' of VARIANT  
EXTERNAL (downstream to 3' end of *cblA* gene) anti-sense  
PCR primers and COMMON INTERNAL sense primers (at 5'  
end of *cblA* gene). Each set specifically amplifies only  
one or another of the 3 known variant *cblA* gene  
10 sequences, including that uniquely associated with the  
Toronto/Edinburgh (T/E) epidemic clone.

- ```

15      common sense primer:
          1
          ATGCT GAAAT ACGTT CCGAT
                                     20**
          variant anti-sense primer:
20      573
          ATGGT TTTTC AGGA GT
                                     558***

```

\*\*\* 558-573: unique sequence beginning 57 bp (base pairs) downstream of 3' end of T/E clone *cblA* gene, i.e. end of *cblA* gene sequence is bp No. 501, thus 501 + 57 = 558.

- 18 -

- To amplify only MS<sub>p</sub>-type\* (non-epidemic) the following primers were used to generate a 573 base pair (bp) PCR product:

common sense primer:

5

1

ATGCT GAAAT ACGTT CCGAT

20\*\*

variant anti-sense primer:

573

10

AGGAT TTCCA AAGGA GT

557\*\*\*

- To amplify only MS<sub>w</sub>-type\*\*\*\* (non-epidemic) the following primers were used to generate a 573 base pair (bp) PCR product:

15

common sense primer:

1

ATGCT GAAAT ACGTT CCGAT

20\*

variant anti-sense primer:

20

573

TGACT TCCGA AGGAC TACT

\* MS<sub>p</sub>-type: non-epidemic, negligible transmissibility *cbIA*<sup>+</sup> strain from Mississippi, for example isolate PC<sub>MS</sub>-2323.

\*\* In accord with conserved region of *cbIA* gene sequence, numbered as Fig. 4.

\*\*\* 557-573: unique sequence beginning 56 bp downstream of 3' end of MS<sub>p</sub>-type *cbIA* gene, i.e. end of *cbIA* gene sequence is bp No. 501, thus 501 + 56 = 557.

\*\*\*\* MS<sub>w</sub>-type: another non-epidemic, negligible transmissibility *cbIA*<sup>+</sup> strain from Mississippi.

**SUBSTITUTE SHEET (RULE 26)**

- 19 -

555\*

**Method B:**

This method was based upon three 'sets' of VARIANT EXTERNAL (downstream to 3' end of *cblA* gene) anti-sense  
5 PCR primers and VARIANT INTERNAL sense primers (at 5' end of *cblA* gene). Each set specifically amplifies only one or another of the 3 known variant *cblA* gene sequences, including that uniquely associated with the Toronto/Edinburgh (T/E) epidemic clone.

- 10 • To amplify only T/E epidemic lineage clones the following primers were used to generate a 331 base pair (bp) PCR product:

variant sense primer:

243

15 GACTG CCCCCG GCTTT GAA

260\*\*

variant anti-sense primer:

573

ATGGT TTTTC AGGAG T

20

558\*\*\*

- To amplify only MS<sub>P</sub>-type (non-epidemic) the following primers were used to generate a 331 base pair PCR product:

---

\* 555-573: unique sequence beginning 54 bp downstream of 3' end of MS<sub>W</sub>-type *cblA* gene, i.e. end of *cblA* gene sequence is bp No. 501, thus 501 + 54 = 555.

\*\* 243-260: in accord with *cblA* gene sequence numbering in Fig. 4 for epidemic isolates PC<sub>T</sub>-5, PC<sub>T</sub>-7, PC<sub>E</sub>-1359, PC<sub>E</sub>-2315.

\*\*\* 558-573: unique sequence beginning 57 bp (base pairs) downstream of 3' end of T/E clone *cblA* gene, i.e. end of *cblA* gene sequence is bp No. 501, thus 501 + 57 = 558.

- 20 -

variant sense primer:

243

GGCCG AGCCG GCGCT GAA

260\*

5

variant anti-sense primer:

573

AGGAT TTCCA AAGGA GT

557\*\*

- 10 • To amplify only MS<sub>W</sub>-type (non-epidemic) the following primers were used to generate a 331 base pair PCR product:

variant sense primer:

243

GACCG CTCCG TCGCT CAA

15

260\*\*\*

variant anti-sense primer:

573

TGACT TCCGA AGGAC TACT

555\*\*\*\*

---

\* 243-260: in accord with *cblA* gene sequence numbering in Fig. 4 for non-epidemic Mississippi isolate PC<sub>MS</sub>-2323.

\*\* 557-573: unique sequence beginning 56 bp downstream of 3' end of MS<sub>P</sub>-type *cblA* gene, i.e., end of *cblA* gene sequence is bp No. 501, thus 501 + 56 = 557.

\*\*\* 243-260: this (MS<sub>W</sub>-type) is a recently discovered variant *cblA* gene sequence carried by another non-epidemic strain of negligible transmissibility from Mississippi.

\*\*\*\* 555-573: unique sequence beginning 54 bp downstream of 3' end of MS<sub>W</sub>-type *cblA* gene, i.e. end of *cblA* gene sequence is bp No. 501, thus 501 + 54 = 555.

**SUBSTITUTE SHEET (RULE 26)**

- 21 -

**Method C:**

This method was based upon three 'sets' of Internal (within *cblA* gene) PCR primers. Each set specifically amplifies only one or another of the 3  
5 known variant *cblA* gene sequences, including that uniquely associated with the T/E epidemic clone.

**METHOD C1:** was based on 'common' sense primer and variant antisense primers:

- To amplify only T/E epidemic lineage ~~clones~~ the following primers were used to generate a 427 base pair (bp) PCR product:

common sense primer:

1

ATGCT GAAAT ACGTT CCGAT

20\*

variant anti-sense primer:

427

CTTCG ACCTT CTTCT GACC

409\*\*

---

\* 1-20: in accord with conserved region of *cblA* gene sequence, numbering as in Fig. 4.

\*\* 427-409: in accord with *cblA* gene sequence numbering in Fig. 4 uniquely for clonal epidemic isolates PC<sub>T</sub>-5, PC<sub>T</sub>-7, PC<sub>E</sub>-1359, PC<sub>E</sub>-2315.

- 22 -

- To amplify only MS<sub>p</sub>-type (non-epidemic) the following primers were used to generate a 427 base pair (bp) PCR product:

common sense primer:

5

1

ATGCT GAAAT ACGTT CCGAT

20\*\*

variant anti-sense primer:

427

10

CAGCG ACAGT TTTCT GGCC

409\*

- To amplify only MS<sub>w</sub>-type (non-epidemic) the following primers were used to generate a 427 base pair (bp) PCR product:

15

common sense primer:

1

ATGCT GAAAT ACGTT CCGAT

20\*\*

variant anti-sense primer:

20

427

CAGCG ACAGT TTTCT GGCC

409\*\*\*

\* 427-409: in accord with *cblA* gene sequence numbering in Fig. 4 uniquely for non-epidemic Mississippi isolate PC<sub>MS</sub>-2323.

\*\* 1-20: in accord with conserved region of *cblA* gene sequence, numbered as in Fig. 4.

\*\*\* 427-409: this (MS<sub>w</sub>-type) is a recently discovered variant *cblA* gene sequence carried by a non-epidemic strain of negligible transmissibility from Mississippi.

- 23 -

METHOD C2: was based on variant sense primers and variant antisense primers:

- To amplify only T/E epidemic lineage clones the following primers were used to generate a 185 base pair (bp) PCR product:

variant sense primer:

243

GACTG CCCC GCTTT GAA

260\*

variant anti-sense primer:

427

CTTCG ACCTT CTTCT GACC

409\*\*

- To amplify only MS<sub>p</sub>-type (non-epidemic) the following primers were used to generate a 185 base pair (bp) PCR product:

variant sense primer:

243

GGCCG AGCCG GCGCT GAA

260\*\*\*

variant anti-sense primer:

427

CTCCG GCCGT CTTCT GTTC

---

\* 243-260: in accord with *cb1A* gene sequence numbering in Fig. 4 for epidemic isolates PC<sub>T</sub>-5, PC<sub>T</sub>-7, PC<sub>E</sub>-1359, PC<sub>E</sub>-2315.

\*\* 427-409: in accord with *cb1A* gene sequence numbering in Fig. 4 for epidemic isolates PC<sub>T</sub>-5, PC<sub>T</sub>-7, PC<sub>E</sub>-1359, PC<sub>E</sub>-2315.

\*\*\* 243-260: in accord with *cb1A* gene sequence numbering in Fig. 4 for non-epidemic Mississippi isolate PC<sub>MS</sub>-2323.

- 24 -

409\*

- To amplify only MS<sub>w</sub>-type (non-epidemic) the following primers were used to generate a 185 base pair (bp) PCR product:

5

variant sense primer:

243

GACCG CTCCG TCGCT CAA

260\*\*

variant anti-sense primer:

10

427

CAGCG ACAGT TTTCT GGCC

409\*\*\*

---

\* 427-409: in accord with *cb1A* gene sequence numbering in Fig. 4 for non-epidemic Mississippi isolate PC<sub>MS</sub>-2323.

\*\* 243-260: this (MS<sub>w</sub>-type) is a recently discovered variant *cb1A* gene sequence carried by another non-epidemic strain of negligible transmissibility from Mississippi.

\*\*\* 427-409: this (MS<sub>w</sub>-type) is a recently discovered variant *cb1A* gene sequence carried by another non-epidemic strain of negligible transmissibility from Mississippi.

**SUBSTITUTE SHEET (RULE 26)**

- 25 -

The probability of the random occurrence of 16- to 19-base long sequences, such as the specific primers employed in any of the above methods, is once every  $4^{16}$  (or ca. one in  $10^9$ ) to  $4^{19}$  (or ca. one in  $10^{11}$ ) bp x 2. Given that the size of the *P. cepacia* genome is ca.  $7 \times 10^6$  bp, the chance occurrence would be remote.

In order to determine whether a given sample contains a transmissible strain of *P. cepacia* any one of the above pairs of primers corresponding to the T/E epidemic lineage is used in standard PCR reactions with DNA from the bacterial strain to be tested.

Following PCR amplification, the reaction products are analyzed for the presence of the appropriate size PCR product using standard methods. For example, in Method A (when the pair of primers for the T/E epidemic lineage is used), the detection of a 573 bp PCR product by electrophoresis on an agarose gel would indicate that the sample contains a strain of the highly transmissible T/E. Whereas, the absence of such a PCR product would indicate otherwise. Similarly in Methods B, C1 and C2, the presence of a strain of the highly transmissible T/E would be confirmed by PCR products of 331 bp, 427 bp and 185 bp, respectively.

- 26 -

Hybridization-specific detection, based on 'probes' derived from unique regions of variant *cblA* gene sequences:

Each unique probe sequence shown below can be generated by restriction endonuclease double digestion of one or another of the three *cblA* gene variants, based on:

(i) common *Sma*I site at bp (base pair) 273-278  
 10 (C CCGGG), flanking 3' end of *cblA* probe

(ii) common *Bsr*I site at bp 371-376 (AAC TGG),  
 flanking 5' end of *cblA* probe

the *cblA*-specific DNA probes:

• Hybridization with only T/E epidemic lineage clones  
 15

Unique T/E *cblA* gene 'probe' sequence:

273  
 C CCGGG CGCGG CGGAA ATTCC GCTGT CGGTC AAGCT  
 TGGCG AAACC GAGCT GACCA CCACG GCCGC GACGC  
 20 TGAAG ACCGC AGAGC TCTTC CCCGG CGAAC TGG  
 376\*

---

\* 273-376: in accord with *cblA* gene sequence numbering in Fig. 4 for epidemic isolates PC<sub>T</sub>-5, PC<sub>T</sub>-7, PC<sub>E</sub>-1359, PC<sub>E</sub>-2315.

- 27 -

- Hybridization with only MS<sub>p</sub>-type (non-epidemic) strains

Unique MSP-type *cb1A* gene 'probe' sequence:

273

5            C CCGGG CGCCA AGGAG ATTCC GCTGG CGGTC AAGCT  
GGGCA CCACC GCGCT GAGCA CCACG GCGAC GACGC  
TGAAG GCGTC GGAGA TCTTT ACCGG CGAAC TGG

376\*

- Hybridization with only MSW-type (non-epidemic) strains

Unique MS<sub>W</sub>-type *cb1A* gene 'probe' sequence:

273

15 C CCGGG TCGGG CCGAA ATTCC GTTGG CTGTC AAAC  
GGGCG ACACC GAGTT GAGCA CGACG TCGGC GACCC  
TGAAG GCCGC GGAAC TCTTT CCCGG CGAAC TGG

376\*\*

The probes for the T/E lineage disclosed above can be used in standard hybridization methods, such as colony hybridization assays or Southern blot transfer, to detect the presence of the highly transmissible *P. cepacia* strain of the T/E lineage.

\* 273-376: in accord with *cblA* gene sequence numbering in Fig. 4 for non-epidemic Mississippi isolate PC<sub>MS</sub>-2323.

\*\* 273-376: this (MS<sub>w</sub>-type) is a recently discovered variant *cblA* gene sequence carried by another non-epidemic strain of negligible transmissibility from Mississippi.

- 28 -

## RESULTS

The above-described methods were used to characterize the epidemiological-relatedness of the 15 Toronto isolates expressing mucin-binding Cbl pili. To  
5 investigate the genetic relationship between these isolates and those found elsewhere, we included clinical and environmental isolates as well as 78 strains from the seven other CF centers cited above that were *cblA*<sup>-</sup>. At the time the report of RFLP-  
10 identical *P. cepacia* isolates transmitted among patients at an Edinburgh CF center appeared and we obtained the involved strains<sup>3</sup> to include in this phylogenetic characterization. See Fig. 1A and 1B. Profiles in lanes 9-17 of both figures depict typical  
15 polymorphic patterns resolved for isolates from different CF centers. For these isolates mean D (Dice coefficient of similarity)<sup>12</sup> for any pair by PFGE-resolved chromosomal macro-RFLP profile was  $0.14 \pm 0.07$  (Fig. 1A), a level of diversity not significantly  
20 different from that found previously among eight independently isolated ATCC environmental and clinical control isolates<sup>9</sup>. A similar degree of chromosomal RFLP variability was found between the other CF-associated isolates from the seven CF centers (results  
25 not shown), confirming that these are epidemiologically distinct strains with RFLP variability not significantly different from that of the random collection of ATCC strains ( $0.1 > p > 0.05$ ). Despite the  
30 lower discriminatory power of ribotyping<sup>9</sup>, a similar degree of phylogenic relationship among these CF-associated isolates is apparent in Fig. 1B.

- 29 -

The heterogeneity of the RFLP profiles of the isolates from the seven CF centers (e.g. lanes 9-17, Fig. 1A and 1B) is similar to that described in our previous study involving multiple isolates from 23 patients at the UNC CF center<sup>9</sup>. This degree of variability contrasts markedly with the two closely-related, conserved RFLP patterns found for the 15 *cbIA*<sup>-</sup> encoding Toronto CF center isolates (lanes 1-4, Fig. 1A and 1B). Here, by examining both PFGE and ribotype RFLP profiles, the coefficient of similarity amongst the Toronto isolates proved to be very high, with PFGE  $D = 0.95 \pm 0.03$  and *rrn*  $D = 0.87 \pm 0.09$ . This contrasted with (a) the mean *D* values amongst isolates from the other seven CF centers which was very low (e.g. lanes 9-17, Fig. 1A and 1B), and (b) the mean *D* between the Toronto isolates and the other CF center isolates which was also very low: PFGE  $D = 0.20 \pm 0.07$ , *rrn*  $D = 0.39 \pm 0.09$ . These findings strongly suggest that all 15 of the Toronto CF center isolates were members of a unique lineage associated with an epidemic.

Displayed in lanes 5-8 of Fig. 1A and 1B are *P. cepacia* PFGE and ribotype RFLP profiles of isolates from CF patients at the Edinburgh CF center<sup>3</sup>. The RFLP profiles displayed in Fig. 1A and 1B support this picture, as *D* for any analyzed pair by either type of RFLP profile was very high (PFGE  $D = 0.98 \pm 0.02$ , *rrn*  $D = 1.0$ ). Further, pair-wise comparison of the Edinburgh strains to the closely-related Toronto CF center strains (lanes 1-4 of Fig. 1A and 1B) likewise produced robust *D* values (PFGE  $D = 0.97 \pm 0.03$ , *rrn*  $D = 0.90 \pm 0.04$ ), strongly suggesting that the Edinburgh and Toronto isolates are members of the same unique lineage, despite the Atlantic Ocean barrier.

- 30 -

Based on ribosomal RNA (*rrn*) RFLP profiles, phylogenetic relationships of the 133 isolates described above and in the Methods section were determined with the neighbor-joining method<sup>13</sup>. Confidence intervals on the tree topology were estimated by bootstrapping analysis<sup>14</sup>. See Fig. 2. The resultant phylogenetic tree indicates that: (i) the cluster of Toronto and Edinburgh isolates comprise a single, clonally-related lineage, only distantly related to all other isolates, and (ii) the remaining, independently isolated strains from other CF centers are as distantly-related to one another as they are either to the Toronto/Edinburgh clusters or to the independently isolated non-CF clinical and environmental strains.

We then examined the epidemic Edinburgh isolates to see if, like the Toronto strains<sup>4</sup>, they expressed Cbl pili and encoded the *cblA* gene. Phenotypic survey using electron microscopy revealed that these highly transmissible strains expressed appendage pili that were structurally equivalent to those expressed by all of the *cblA*<sup>+</sup> Toronto isolates<sup>4, 11</sup>. See Fig. 3. Genotypic survey was carried out by stripping *rrn*-probe from an *EcoRI* chromosomal digest membrane (Fig. 1B) followed by hybridization with a *cblA* probe. Fig. 1C (lanes 1-8) indicates that four highly transmissible Edinburgh isolates as well as the closely-related Toronto clones encode *cblA*.

Because the implications of our studies have the potential to directly affect clinical management of some 70,000 CF patients in Europe and North America, we used DNA sequence analysis to test the RFLP-based conclusions that isolates from Toronto and Edinburgh were clonal.

SUBSTITUTE SHEET (RULE 26)

- 31 -

Primers were synthesized from the *cblA* sequence of the Toronto isolate PC<sub>T</sub>-74 and used for PCR-based amplification of the *cblA* gene from isolates to be characterized. Resultant PCR products were then  
5 cloned and sequenced (see Methods). Complete *cblA* sequences were thus obtained from isolates with the two slightly variant though closely-related RFLP profiles typical of the 15 Toronto CF center isolates (Fig. 1A and 1B, lanes 1-4), the two slightly variant though  
10 closely-related RFLP profiles typical of the 13 Edinburgh CF center isolates (Fig. 1A and 1B, lanes 5-8), and the significantly variant Jackson Mississippi CF-associated strain PC<sub>MS</sub>-2323 (Fig. 1A and 1B, lane 17). Comparison of these five sequences indicated  
15 that the chromosomally-encoded, 501 bp *cblA* pilin subunit structural gene carried by the closely-related isolates from the Toronto and Edinburgh CF centers was invariant in sequence. In contrast, the *cblA* gene encoded by the distantly-related Jackson Mississippi  
20 strain PC<sub>MS</sub>-2323 exhibited polymorphism at the sequence level, with changes in 60 bp of the 501 bp sequence (88% identity; see Figure 4). The perfect conservation of the *cblA* pilin sequence among isolates from multiple patients over four years at the Toronto and Edinburgh  
25 centers is precisely what would be expected for epidemic transmission of a highly infectious clone. Likewise, variability of *cblA* encoded by the Mississippi CF center isolate is in accord with that expected for a distantly-related isolate.

30

## CONCLUSIONS

Consideration of these findings leads us to conclude: (i) isolates of *P. cepacia* are not equally

- 32 -

transmissible between CF patients; rather, there exists at least one significantly divergent, highly transmissible clonal lineage plus numerous moderately heterogeneous lineages of negligible transmissibility (see Fig. 2), and (ii) the highly transmissible lineage identified is responsible for epidemics at North American and British CF centers. This was most likely due to an as yet unidentified transatlantic transmission, possibly summer camp attendance.

Based solely on *rrn* RFLP profiles or anecdotal evidence, additional reports exist suggesting the occurrence of *P. cepacia* transmission at CF centers in Philadelphia<sup>2</sup> and Cleveland<sup>17</sup>, respectively. We characterized strains involved with both of these putative outbreaks (see Methods section) and found that by neither ribotype or macro-chromosomal RFLPs profile did the prototypic RFLP fingerprints of the putatively epidemic strains from either center appear similar to one another (mean  $D \leq 0.3$ ), nor to the unique, highly transmissible lineage involved with the Toronto and Edinburgh CF patients. Nonetheless, highly conserved RFLP profiles (mean  $D \geq 0.85$ ) within the individual outbreaks did support a picture of epidemic transmission within each of the two centers. When these isolates were further characterized, hybridization-based survey for the presence of the *cbIA* pilin gene proved negative for all 35 of the involved strains. These results suggest that there may exist *P. cepacia* lineages of high transmissibility other than the *cbIA*<sup>+</sup> clone that we have identified.

While we have hereinbefore described a number of embodiments of this invention, it is apparent that our basic disclosures can be altered to provide other

**SUBSTITUTE SHEET (RULE 26)**

- 33 -

embodiments which utilize the methods and compositions of this invention.

To the extent the following references include protocols or materials employed in the methods discussed herein, they are incorporated herein by reference.

1. LiPuma, J., Mortensen, J., Dasen, S. & Stull, T. Ribotype analysis of *P. cepacia* from cystic fibrosis treatment centers. J. Pediatrics. 113, 859-862 (1988).
2. LiPuma, J., Dasen, S., Nielson, D., Stern, R. & Stull, T. Person-to-person transmission of *P. cepacia* between patients with cystic fibrosis. Lancet. 336, 1094-1096 (1990).
3. Govan, J. R. W. et al. Evidence for transmission of *Pseudomonas cepacia* by social contact in cystic fibrosis. Lancet. 342, 15-19 (1993).
4. Sajjan, S. et al. Cable (Cbl) type II pili of cystic fibrosis-associated *Burkholderia* (*Pseudomonas*) *cepacia*: nucleotide sequence of the *cblA* major subunit pilin gene and novel morphology of the assembled appendage fibers. J. Bacter. 177, 1030-1038 (1995).
5. Tablan, O. et al. *Pseudomonas cepacia* colonization in patients with cystic fibrosis: Risk factors and clinical outcome. J. Pediatr. 107, 382-387 (1985).
6. Thomassen, M. J., Demko, C., Klinger, J. & Stern, R. *Pseudomonas cepacia* colonization among patients with cystic fibrosis - A new opportunist. Am. Rev. Respir. Dis. 131, 791-796 (1985).

- 34 -

7. Ramirez, J. C., et al. Bilateral lung transplantation for cystic fibrosis. J. Thor. Cardiovasc. Surg. 103, 287-294 (1992).
8. Snell, G. I., deHoyos, A., Krajden, M., Winton, T.  
5 & Maurer, J.R. *Pseudomonas cepacia* in lung transplant recipients with cystic fibrosis. Chest. 103, 466-471 (1993).
9. Steinbach, S. et al. Transmissibility of  
10 *Pseudomonas cepacia* infection in clinic patients and lung-transplant recipients with cystic fibrosis. The New England J. of Med. 331, 981-987 (1994).
10. Anderson, D. J., Kuhns, J.S., Vasil, M.L.,  
15 Gerding, D.N. & Janoff, E.N. DNA fingerprinting by pulsed field gel electrophoresis and ribotyping to distinguish *Pseudomonas cepacia* isolates from a nosocomial outbreak. J. Clin. Microbiol. 29, 648-649 (1991).
11. Goldstein, R. et al. Structurally variant classes  
20 of pilus appendage fibers coexpressed from Burkholderia (*Pseudomonas*) *cepacia*. J. Bacteriol. 177, 1039-1052 (1995).
12. Dice, L. Measures of the amount of ecological  
25 association between species. Ecology. 26, 297-302 (1945).
13. Saitou, N. & Nei, M. The neighbor-joining method: A new method for reconstructing phylogenetic trees. Mol. Biol. Evol. 4, 406-425 (1987).
14. Felsenstein, J. Confidence limits on phylogenies:  
30 an approach using the bootstrap. Evolution. 39, 783-791 (1985).
15. Nelson, K. & Selander, R. Evolutionary genetics of the proline permease (putP) and the control region

- 35 -

- of the proline utilization operon in populations of *Salmonella* and *E. coli*. *J. Bacteriol.* 174, 6886-6895 (1992).
16. Smith, N. & Selander, R. Sequence invariance of the antigen-coding central region of the phase 1 flagellar filament gene (*fliC*) among strains of *Salmonella typhimurium*. *J. Bacteriol.* 172, 603-609 (1990).
17. Thomassen, M. J., C. Demko, C. Doershuk, R. C. Stern & J. Klinger. *Pseudomonas cepacia*: Decrease in colonization in patients with cystic fibrosis. *Am. Rev. Respir. Dis.* 134, 669-671 (1986).
18. Sajjan, U., Corey, M., Karmali, M. & Forstner, J. Binding of *Pseudomonas cepacia* to normal human intestinal mucin and respiratory mucin from patients with cystic fibrosis. *J. Clin. Invest.* 89, 648-556 (1992).
19. Sajjan, S. & Forstner, J. Identification of the mucin-binding adhesin of *P. cepacia* isolated from patients with cystic fibrosis. *Infect. Immun.* 60, 1434-1440 (1992).
20. Vinograd, J. & Hearst, J.E. Equilibrium sedimentation of macromolecules and viruses in a density gradient. *Fortschritte der Chemie Organischer Naturstoffe*. XX, 372-422 (1962).
21. Mullis, K. & Faloona, F. Specific synthesis of DNA in vitro via a polymerase-catalyzed chain reaction. *Meth. Enzymol.* 155, 335-350 (1987).
22. Sanger, F., Nicklen, S. & Coulson, A. DNA sequencing with chain-terminating inhibitors. *Proc. Natl. Acad. Sci. (USA)*. 74, 5463-5467 (1977).

- 36 -

23. Prevost, G., Jaulhac, B. & Piemont, Y. DNA fingerprinting by PFGE is more effective than ribotyping in distinguishing among MRSA isolates. J. Clin. Microbiol. 30, 967-973 (1992).
- 5 24. Rabkin, C., Jarvis, W., Anderson, R., Stull, T. & Woods, D. P. *cepacia* typing systems: Collaborative study to assess their potential in epidemiologic investigations. Rev. Infect. Dis. 11, 600-607 (1989).
- 10 25. Nei, M. & Li, W. H. Mathematical model for studying genetic variation in terms of restriction endonucleases. Proc. Natl. Acad. Sci. USA. 76, 5269-5273 (1979).
- 15 26. Arthur, M. et al. Restriction fragment length polymorphisms among uropathogenic *Escherichia coli*: pap-related sequences compared with *rrn* operons. Infect. Immun. 58, 471-479 (1990).
- 20 27. Arthur, M. et al. Molecular epidemiology of adhesin and hemolysin virulence factors among uropathogenic *Escherichia coli*. Infect. Immun. 57, 303-313 (1989).
- 25 28. Berg, K., Squires, C. & Squires, C. *In vivo* translation of a region within the *rrnB* 16S rRNA gene of *Escherichia coli*. J. Bacteriol. 169, 1691-1701 (1987).
29. Birnboim, H. A rapid alkaline extraction method for the isolation of plasmid DNA. Methods Enzymol. 100, 243-255 (1983).
- 30 30. Feinberg, A. & Vogelstein, B. A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. Anal. Biochem. 137, 266-267.

**SUBSTITUTE SHEET (RULE 26)**

- 37 -

## We Claim:

1. A method for detecting in a sample the presence of a strain of a transmissible lineage of *Pseudomonas cepacia* comprising the step of:  
5 analyzing the sample for restriction fragment length polymorphisms (RFLPs) linked to a strain known to be of a transmissible lineage of *Pseudomonas cepacia*.
2. The method according to claim 1 wherein  
10 the sample is analyzed by ribotyping.
3. The method according to claim 1 wherein the sample is analyzed by DNA fingerprinting using pulsed field gel electrophoresis.
4. The method according to any one of  
15 claims 1-3 wherein the strain known to be of a transmissible lineage of *Pseudomonas cepacia* expresses cable adhesin type II pili.
5. The method according to claim 4 wherein the strain of *Pseudomonas cepacia* is of the highly  
20 transmissible Toronto/Edinburgh lineage.
6. The method according to claim 5 wherein the strain is selected from the group consisting of: PC<sub>T</sub>-5, PC<sub>T</sub>-7, PC<sub>T</sub>-19, PC<sub>T</sub>-25, PC<sub>E</sub>-509, PC<sub>E</sub>-1359, PC<sub>E</sub>-1392 and PC<sub>E</sub>-2315.
7. A method for detecting in a sample the presence of a strain of a transmissible lineage of *Pseudomonas cepacia* by polymerase chain reaction (PCR)

- 38 -

using one or more pairs of oligonucleotide primers,  
 said primers having nucleotide sequences identical to  
 portions of a gene encoding a 17 kDa major subunit  
 pilin protein of the cable adhesin type II<sub>P</sub>. *cepacia*  
 5 pili.

8. A method for detecting in a sample the  
 presence of a strain of a transmissible lineage of  
*Pseudomonas cepacia* by polymerase chain reaction (PCR)  
 using one or more pairs of oligonucleotide primers  
 10 selected from the group consisting of:

(a) common sense primer:

1

ATGCT GAAAT ACGTT CCGAT

20

15 variant anti-sense primer:

573

ATGGT TTTTC AGGA GT

558;

(b) variant sense primer:

20

243

GACTG CCCC GCTTT GAA

260

variant anti-sense primer:

573

25

ATGGT TTTTC AGGAG T

558;

- 39 -

(c) common sense primer:

1

ATGCT GAAAT ACGTT CCGAT

20

5

variant anti-sense primer:

427

CTTCG ACCTT CTTCT GACC

409; and

(d) variant sense primer:

10

243

GACTG CCCC GCTTT GAA

260

variant anti-sense primer:

427

15

CTTCG ACCTT CTTCT GACC

409.

9. A method for detecting in a sample the presence of a strain of a transmissible lineage of *Pseudomonas cepacia* by DNA hybridization using a DNA probe having the DNA sequence:

20

273

C CCGGG CGCGG CGGAA ATTCC GCTGT CGGTC AAGCT

TGGCG AAACC GAGCT GACCA CCACG GCCGC GACGC

TGAAG ACCGC AGAGC TCTTC CCCGG CGAAC TGG

25

376.

- 40 -

10. A pair of oligonucleotide primers  
selected from the group consisting of:

(a) common sense primer:

1

5 ATGCT GAAAT ACGTT CCGAT

20

variant anti-sense primer:

573

ATGGT TTTTC AGGA GT

10

558;

(b) variant sense primer:

243

GACTG CCCCCG GCTTT GAA

260

15

variant anti-sense primer:

573

ATGGT TTTTC AGGAG T

558;

(c) common sense primer:

1

20

ATGCT GAAAT ACGTT CCGAT

20

variant anti-sense primer:

427

25

CTTCG ACCTT CTTCT GACC

409; and

SUBSTITUTE SHEET (RULE 26)

- 41 -

- (d) variant sense primer:  
243  
GACTG CCCCC GCTTT GAA  
260  
5 variant anti-sense primer:  
427  
CTTCG ACCTT CTTCT GACC  
409.

11. A pair of oligonucleotide primers  
10 selected from the group consisting of: \_\_\_\_\_

- (a) common sense primer:  
1  
ATGCT GAAAT ACGTT CCGAT  
20  
15 variant anti-sense primer:  
573  
AGGAT TTCCA AAGGA GT  
557;
- (b) common sense primer:  
1  
20 ATGCT GAAAT ACGTT CCGAT  
20  
variant anti-sense primer:  
573  
25 TGACT TCCGA AGGAC TACT  
555;
- (c) variant sense primer:  
243  
GGCCG AGCCG GCGCT GAA  
30 260

- 42 -

variant anti-sense primer:

573

AGGAT TTCCA AAGGA GT

557;

5

(d)

variant sense primer:

243

GACCG CTCCG TCGCT CAA

260

variant anti-sense primer:

10

573

TGACT TCCGA AGGAC TACT

555;

(e)

common sense primer:

1

15

ATGCT GAAAT ACGTT CCGAT

20

variant anti-sense primer:

427

CTCCG GCCGT CTTCT GTTC

20

409;

(f)

common sense primer:

1

ATGCT GAAAT ACGTT CCGAT

20

25

variant anti-sense primer:

427

CAGCG ACAGT TTTCT GGCC

409;

**SUBSTITUTE SHEET (RULE 26)**

- 43 -

(g) variant sense primer:

243

GGCCG AGCCG GCGCT GAA

260

5

variant anti-sense primer:

427

CTCCG GCCGT CTTCT GTTC

409; and

10

(h) variant sense primer:

243

GACCG CTCCG TCGCT CAA

260

variant anti-sense primer:

15

427

CAGCG ACAGT TTTCT GGCC

409.

20

12. A DNA probe having the DNA sequence:

273

C CCGGG CGCGG CGGAA ATTCC GCTGT CCGTC AAGCT

TGGCG AAACC GAGCT GACCA CCACG GCCGC GACGC

TGAAG ACCGC AGAGC TCTTC CCCGG CGAAC TGG

376.

25

13. A DNA probe having a DNA sequence  
selected from the group consisting of:

- 44 -

- (a) C CCGGG CGCCA AGGAG ATTCC GCTGG CGGTC AAGCT  
GGGCA CCACC GCGCT GAGCA CCACG GCGAC GACGC  
TGAAG GCGTC GGAGA TCTTT ACCGG CGAAC TGG; and
- (b) C CCGGG TCGGG CCGAA ATTCC GTTGG CTGTC AAAC  
GGGCG ACACC GAGTT GAGCA CGACG TCGGC GACCC  
TGAAG GCCGC GGAAC TCTTT CCCGG CGAAC TGG.
14. A diagnostic kit comprising at least one pair of oligonucleotide primers of claim 10.
15. A diagnostic kit comprising the DNA probe of claim 12.
16. An isolated DNA molecule encoding the 17 kDa major subunit pilin protein of the cable adhesin type II<sub>P</sub>. *cepacia* pilus.
17. The DNA molecule according to claim 15 having a DNA sequence selected from the two DNA sequences depicted in Figure 4.
18. A recombinant DNA molecule comprising a DNA molecule of claim 16.
19. A unicellular host transformed with a recombinant DNA molecule according to claim 18.
20. A method of producing a polypeptide comprising the step of culturing a unicellular host transformed with a recombinant DNA molecule according to claim 18.

**SUBSTITUTE SHEET (RULE 26)**

- 45 -

21. A polypeptide produced according to the method of claim 20.

22. An antibody directed against a polypeptide of claim 21.

5           23. A diagnostic kit comprising a polypeptide of claim 21.

24. A diagnostic kit comprising an antibody of claim 22.

1/6.

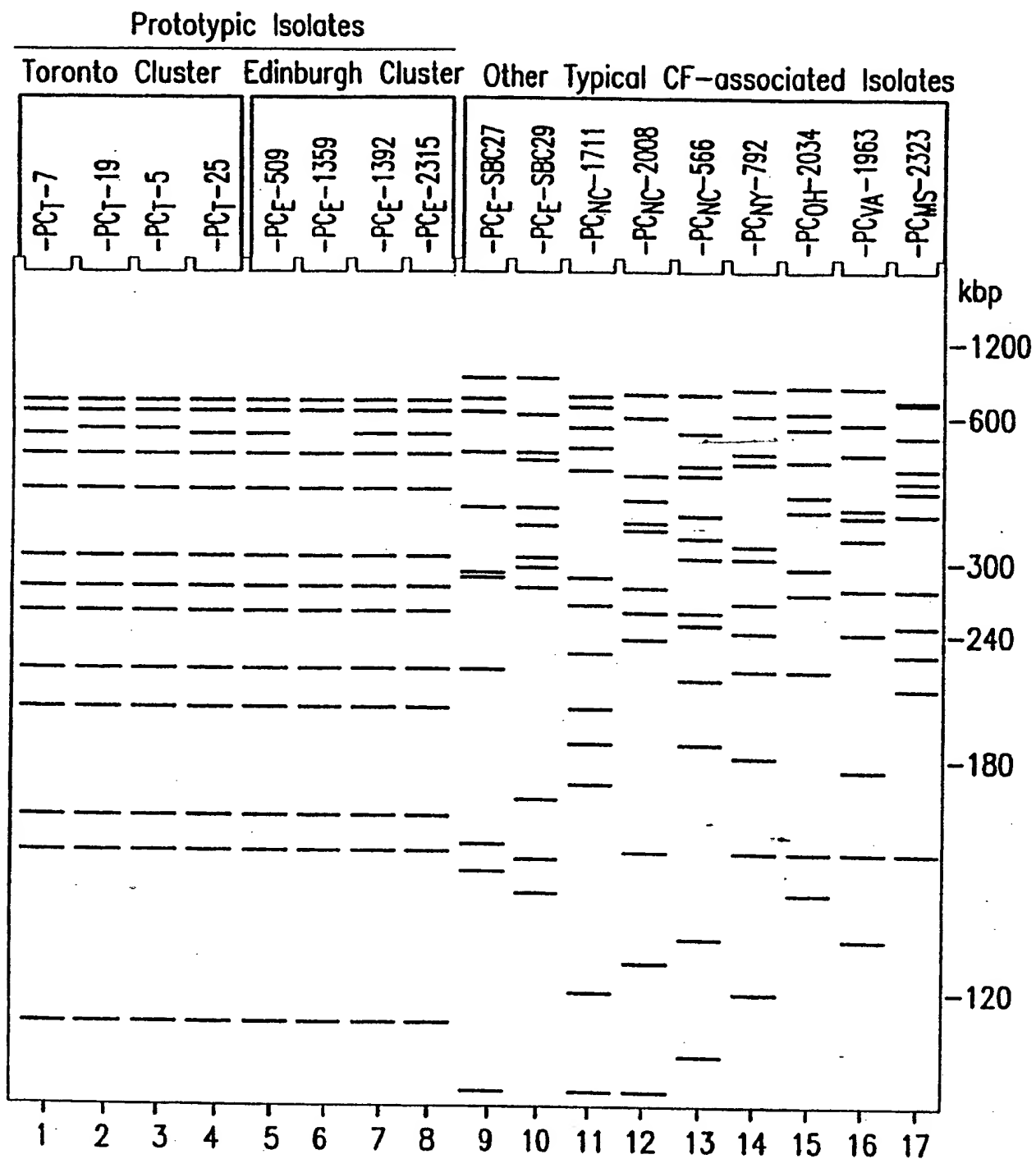


FIG.1A

**SUBSTITUTE SHEET (RULE 26)**

2/6

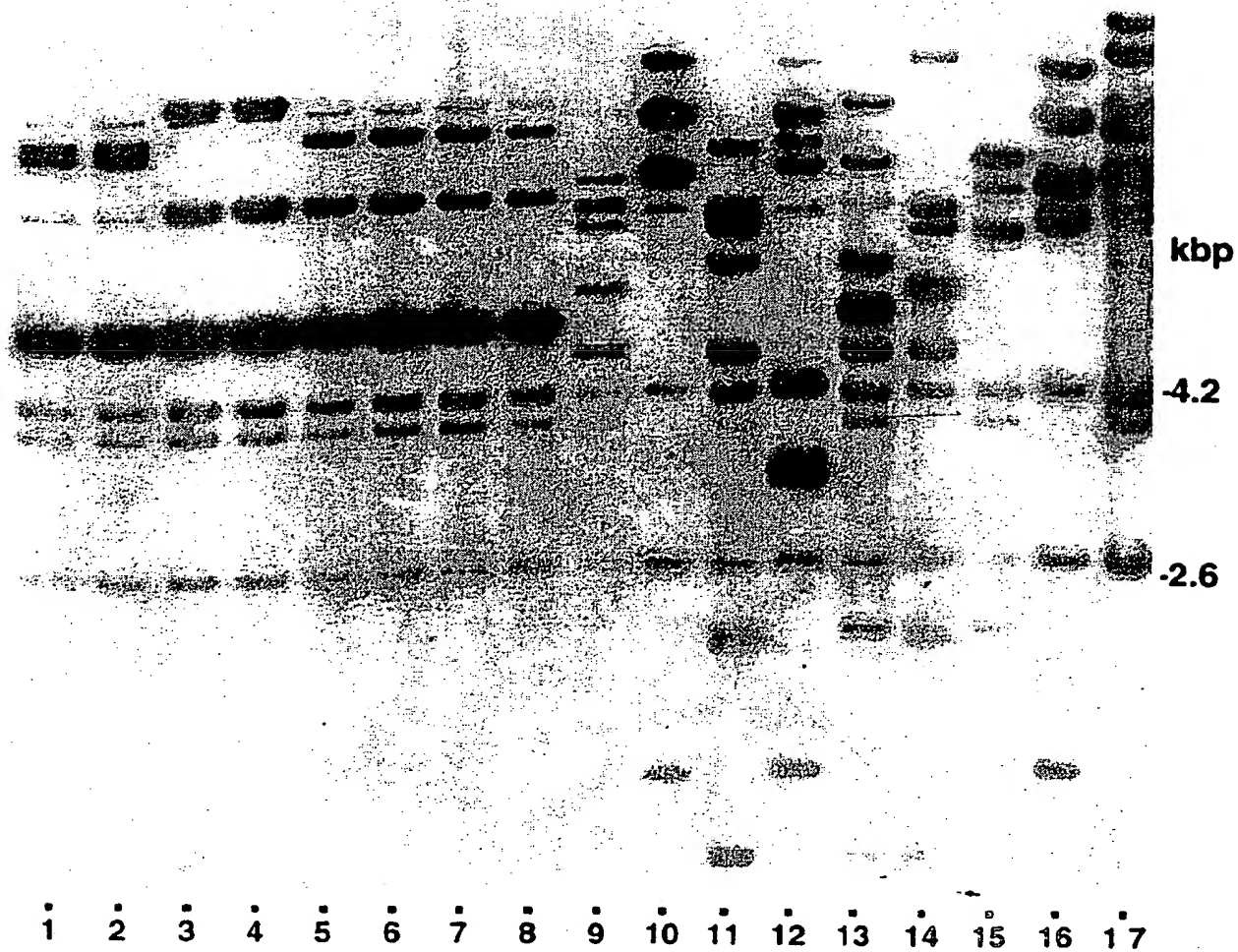


FIG. 1B

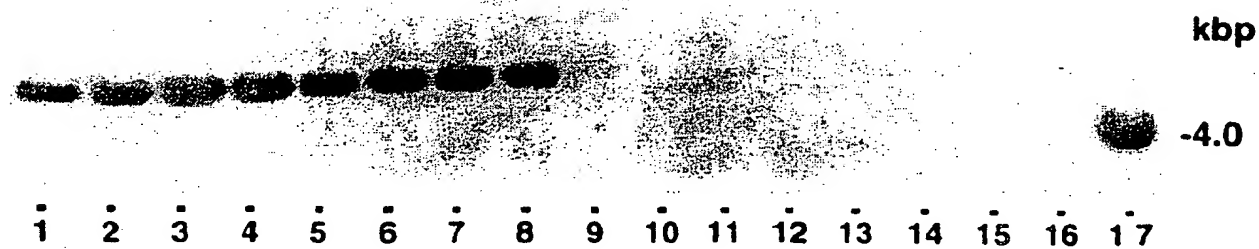


FIG. 1C

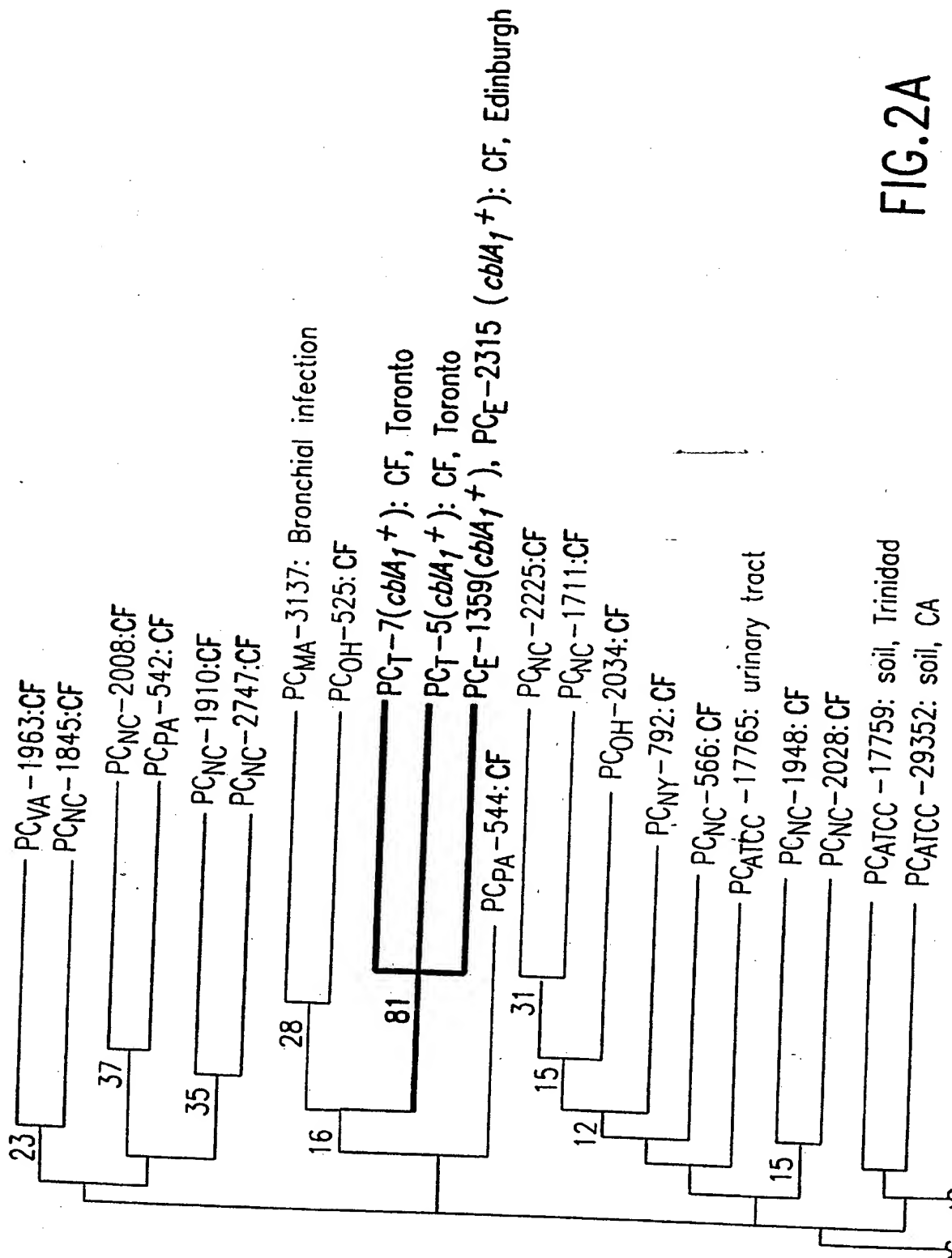


FIG.2A

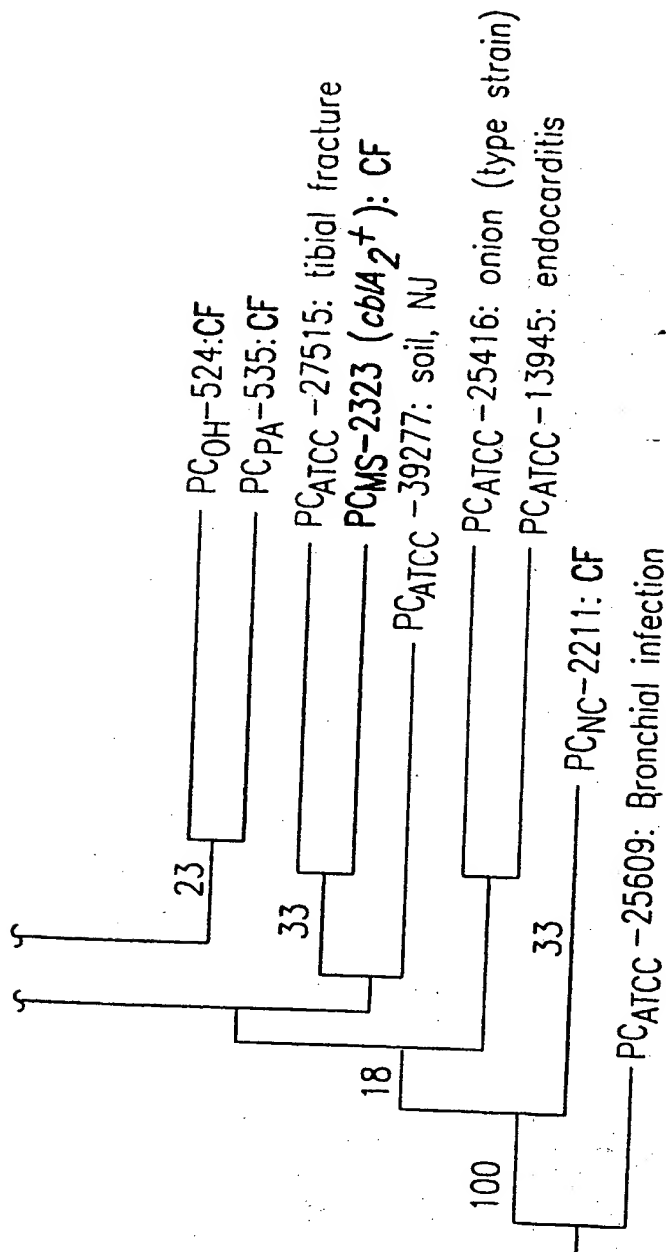


FIG.2B

5/6



FIG. 3

6/6

```
1 ATGCTGAAATACGTTCCGATCGCTGCTGCTCTGATGTTCGATGTTCGGC 50  
| | | | | | | | | | | | | | | | | | | | | |  
1 ATGCTGAAATACGTTCCGATCCGTGCCGCCGCCCTGATGTTCGATGTTCGGC 50  
  
51 TTACGCCGTCAGGAAGGACATTACCGTCAACGCCAACGTCGACACGACGC 100  
| | | | | | | | | | | | | | | | | | | | | |  
51 TCACGCCGTCAGGAAGGACATCACCGTCAACGCCAACGTCGACACGACGC 100  
  
101 TCGAAATGCTGTTCGGCGGACGGCTCGGCACTGCCGACGACCATGCAGATG 150  
| | | | | | | | | | | | | | | | | | | | | |  
101 TCGAGATGCTGTTCGGCGGACGGTTCGGCGCTGCCGACGACCATGCAGATG 150  
  
151 CAATATCTGCCGGGTACGGGTCTTCAGGCAGCTGTAGTGAACACGAAGAT 200  
| | | | | | | | | | | | | | | | | | | | | |  
151 CAGTATCTGCCGGGTCTCGGTCTCCAGGCGGCTGAAGTGAACACGAAGAT 200  
  
201 C TTCACGAACGACAAGGCAAAGGATCTGCAGATCCGCCTCGCGACTGCCC 250  
| | | | | | | | | | | | | | | | | | | | | |  
201 C TTCACGAACGACAAGGCGAAGGATCTGCAGATCCGCCTCGCGGCCGAGC 250  
  
251 CGGCTTTGAAGAACCAGACGAGCCCCGGGCGCGGCGGAAATTCCGCTGTTCG 300  
| | | | | | | | | | | | | | | | | | | | | |  
251 CGGCGCTGAAGAACCAGACGAGCCCCGGGCGCCAAGGAGATTCCGCTGGCG 300  
  
301 GTCAAGCTTGCGGAAACCGAGCTGACCACCACGGCCGCGACGCTGAAGAC 350  
| | | | | | | | | | | | | | | | | | | | | |  
301 GTCAAGCTGGGCACCACCGCGCTGAGCACCACGGCGACGACGCTGAAGGC 350  
  
351 CGCAGAGCTCTTCCCCGGCGAACTGGCACAAAGGTTCTGAACGTGCTGGCGC 400  
| | | | | | | | | | | | | | | | | | | | | |  
351 GTCGGAGATCTTTACCGGTGAACTGGCGCAGGGCTCGAACGTGCTGCCGC 400  
  
401 TGTCGATCGGTTCAGAAGAAGGTCTGAAGCCGTCACGGCGTCCGGCAGCTAC 450  
| | | | | | | | | | | | | | | | | | | | | |  
401 TGTCGATCGAACAGAAGACGGCCGGAGCCATCACGGCTGCCGGCAGCTAT 450  
  
451 CAGGGCCTCGTCAAGCGTGATCGTCACGCAGAGCGCGGCTTCGGGTAGCTA 500  
| | | | | | | | | | | | | | | | | | | | | |  
451 CAAGGCCTCGTCAAGCGTGATCGTCACGCAGAGCGCGCATCGGGTAGCTA 500  
  
501 A 501  
|  
501 A 501
```

FIG. 4

**SUBSTITUTE SHEET (RULE 26)**



## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

|                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |           |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |
|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>(51) International Patent Classification 6 :</b><br><b>C12Q 1/68, C12N 15/31, C07K 14/21, 16/12</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 | <b>A2</b> | <b>(11) International Publication Number:</b> <b>WO 97/01647</b><br><b>(43) International Publication Date:</b> 16 January 1997 (16.01.97)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       |
| <b>(21) International Application Number:</b> PCT/US96/11132<br><b>(22) International Filing Date:</b> 28 June 1996 (28.06.96)<br><b>(30) Priority Data:</b><br>60/000,828 28 June 1995 (28.06.95) US<br><b>(71) Applicant:</b> TRUSTEES OF HEALTH & HOSPITALS OF THE CITY OF BOSTON, INC. [US/US]; 6th floor, 1010 Massachusetts Avenue, Boston, MA 02119 (US).<br><b>(72) Inventor:</b> GOLDSTEIN, Richard; 12 Howland Street, Cambridge, MA 02138 (US).<br><b>(74) Agents:</b> PIERRI, Margaret, A. et al.; Fish & Neave, 1251 Avenue of the Americas, New York, NY 10020 (US).                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |           | <b>(81) Designated States:</b> AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).<br><br><b>Published</b><br><i>Without international search report and to be republished upon receipt of that report.</i> |
| <b>(54) Title:</b> DNA SEQUENCES FOR IDENTIFYING HIGHLY TRANSMISSIBLE LINEAGES OF PSEUDOMONAS (BURKHOLDE-RIA) CEPACIA                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |           |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |
| <b>(57) Abstract</b><br><br><p>The present invention provides DNA based fingerprints and DNA sequences for indentifying highly transmissible lineages of <i>Pseudomonas cepacia</i>. More specifically, the present invention provides genetic band patterns or DNA based fingerprints for identifying highly transmissible lineages of <i>Pseudomonas cepacia</i> produced by: (a) ribotyping analyses, i.e., the determination of restriction fragment length polymorphisms (RFLPs) associated with the multicopy RNA operon (rm); and (b) pulsed field gel electrophoresis (PFGE)-based resolution of chromosomal macro-RFLP patterns. Also provided are unique primer oligonucleotide sequences and DNA probes derived from variants of a gene, encoding a 17-KDa major subunit pilin protein (cb1A) of a cystic fibrosis-associated <i>Pseudomonas cepacia</i>. The invention also discloses methods and diagnostic kits for identifying highly transmissible lineages of <i>Pseudomonas cepacia</i> that utilize the above DNA based fingerprints, oligonucleotide primers and DNA probes. Another object of this invention is to provide isolated DNA molecules encoding a 17-KDa major subunit pilin protein of certain cystic fibrosis-associated <i>Pseudomonas cepacia</i> strains, as well as recombinant DNA molecules, transformed hosts and methods for the production of that protein. Also contemplated are antibodies to the 17-KDa major subunit pilin protein.</p> |           |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |

**FOR THE PURPOSES OF INFORMATION ONLY**

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

|    |                          |    |                                          |    |                          |
|----|--------------------------|----|------------------------------------------|----|--------------------------|
| AM | Armenia                  | GB | United Kingdom                           | MW | Malawi                   |
| AT | Austria                  | GE | Georgia                                  | MX | Mexico                   |
| AU | Australia                | GN | Guinea                                   | NE | Niger                    |
| BB | Barbados                 | GR | Greece                                   | NL | Netherlands              |
| BE | Belgium                  | HU | Hungary                                  | NO | Norway                   |
| BF | Burkina Faso             | IE | Ireland                                  | NZ | New Zealand              |
| BG | Bulgaria                 | IT | Italy                                    | PL | Poland                   |
| BJ | Benin                    | JP | Japan                                    | PT | Portugal                 |
| BR | Brazil                   | KE | Kenya                                    | RO | Romania                  |
| BY | Belarus                  | KG | Kyrgyzstan                               | RU | Russian Federation       |
| CA | Canada                   | KP | Democratic People's Republic<br>of Korea | SD | Sudan                    |
| CF | Central African Republic | KR | Republic of Korea                        | SE | Sweden                   |
| CG | Congo                    | KZ | Kazakhstan                               | SG | Singapore                |
| CH | Switzerland              | LI | Liechtenstein                            | SI | Slovenia                 |
| CI | Côte d'Ivoire            | LK | Sri Lanka                                | SK | Slovakia                 |
| CM | Cameroon                 | LR | Liberia                                  | SN | Senegal                  |
| CN | China                    | LT | Lithuania                                | SZ | Swaziland                |
| CS | Czechoslovakia           | LU | Luxembourg                               | TD | Chad                     |
| CZ | Czech Republic           | LV | Latvia                                   | TG | Togo                     |
| DE | Germany                  | MC | Monaco                                   | TJ | Tajikistan               |
| DK | Denmark                  | MD | Republic of Moldova                      | TT | Trinidad and Tobago      |
| EE | Estonia                  | MG | Madagascar                               | UA | Ukraine                  |
| ES | Spain                    | ML | Mali                                     | UG | Uganda                   |
| FI | Finland                  | MN | Mongolia                                 | US | United States of America |
| FR | France                   | MR | Mauritania                               | UZ | Uzbekistan               |
| GA | Gabon                    |    |                                          | VN | Viet Nam                 |